Litton BIONETICS

Mutagenicity Evaluation of L-Glutamic Acid, HCL FDA 75-59-Final Report-4/77

MUTAGENICITY EVALUATION OF L-GLUTAMIC ACID, HCL FDA 75-59

FINAL REPORT

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MUTAGENICITY EVALUATION
OF
L-GLUTAMIC ACID, HCL
FDA 75-59

FINAL REPORT

SUBMITTED TO

FOOD AND DRUG ADMINISTRATION
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Litton BIONETICS

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EVALUATION SUMMARY

The test compound L-Glutamic Acid, HCL, FDA 75-59, 000138-15-8, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: November 24, 1976

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound FDA 75-59, L-Glutamic Acid, HCL

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: September 3, 1976

2. Description: White cyrstalline powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-59
L-Glutamic Acid, HCL

2. Test solvent: Saline

3. Solubility of the test compound under treatment conditions:
Soluble

4. Additional comments: White crystalline powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: September 8, 1976

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	0.00625	0.7
1/2 50% Survival	0.01250	1.4
50% Survival	0.02500	2.8

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000138158

B. TEST DATE: OCT. 12, 1976

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	31	23	31	19	19	18	22	21	201	248
POSITIVE CONTROL**		---	---	>1000	>1000	895	461	>1000	>1000	>1000	>1000	>1000	>1000
TEST	0.02500 %	---	---	25	19	14	19	23	9	27	27	286	236
	0.01250 %	---	---	21	24	11	10	12	13	27	31	191	210
	0.00625 %	---	---	28	23	18	20	15	13	29	31	197	174
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	25	40	20	12	22	23	24	19	111	123
		RAT	LIVER	20	20	14	11	32	28	40	59	89	77
		MONKEY	LIVER	16	41	12	6	22	36	51	60	57	71
POSITIVE CONTROL***		MOUSE	LIVER	202	154	303	516	>1000	>1000	167	129	123	100
		RAT	LIVER	94	91	>1000	127	462	500	239	173	154	181
		MONKEY	LIVER	513	375	80	119	>1000	>1000	142	183	167	285
TEST	0.02500 %	MOUSE	LIVER	32	46	17	21	17	11	29	34	126	149
	0.01250 %	MOUSE	LIVER	31	28	23	12	9	14	41	39	144	137
	0.00625 %	MOUSE	LIVER	26	27	18	23	17	18	25	34	135	134
	0.02500 %	RAT	LIVER	11	15	14	17	10	10	63	36	79	47
	0.01250 %	RAT	LIVER	16	15	19	11	16	24	49	39	60	65
	0.00625 %	RAT	LIVER	12	28	19	24	12	18	43	52	67	73
	0.02500 %	MONKEY	LIVER	35	58	8	16	15	15	50	60	79	70
	0.01250 %	MONKEY	LIVER	32	70	12	8	18	25	61	47	62	78
	0.00625 %	MONKEY	LIVER	39	30	18	7	16	19	66	55	61	79

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 9M 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/15/76

SPECIES / NONACTIVATION COMPOUND 000138158

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		66.63	18.29	14.48	1.21	13.86	22.36	9.75	CONTROLS
NAP		729.93	4938.27	95.62	143.90	820.41	68.71	38.10	
NA1		67.92	7.95	3.20	1.78	4.50	17.93	8.48	TEST DATA
NA2		68.86	10.00	9.06	1.26	7.38	25.29	10.18	
NA3		65.57	11.21	6.86	1.66	6.33	9.97	22.76	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/15/76

SPECIES ICRFLO/MOUSE COMPOUND 000138158

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	20.58	7.58	2.69	29.04		4.72	23.27	8.14	
ACT	A-C	22.30	5.57	5.85	28.54		3.09	26.15	7.98	
ACT	ALI	24.42	6.44	6.95	49.71	7.94	9.94	26.53	8.68	NEGATIVE CONTROLS
ACT	ALU	21.77	7.57	3.65	25.54	12.18	5.41	28.89	8.63	
ACT	PLI	70.14	136.26	142.77	202.67		106.30	67.69	27.36	POSITIVE CONTROLS
ACT	PLU	22.31	27.00	2.14	38.36		110.39	32.40	11.43	
ACT	LI1	28.48	12.90	6.87	74.35	11.15	11.73	26.80	12.01	
ACT	LI2	21.65	5.19	5.58	29.40		8.46	26.80	11.64	
ACT	LI3	26.77	9.36	2.79	28.41		11.00	24.68	11.03	TEST DATA
ACT	LU1	22.69	16.67	6.16	30.44		9.70	28.87	10.56	
ACT	LU2	19.89	5.46	7.62	29.21		9.23	24.47	9.40	
ACT	LU3	18.81	6.16	7.04	52.58		7.74	29.48	11.13	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/15/76

SPECIES SPRDAW/RAT COMPOUND 000138158

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	20.72	10.78	4.17	4.92	15.89		43.00	21.56	
ACT	A-C	25.83	10.16	2.65	4.12	12.44		51.25	20.37	NEGATIVE CONTROLS
ACT	ALI	31.45	13.54	3.74	18.45	11.62	16.09	41.81	26.41	
ACT	ALU	27.04	12.64	2.11	7.49	20.06	15.18	39.12	21.07	
ACT	PLI	61.73	246.86	122.50	218.59	84.05		79.89	60.49	POSITIVE CONTROLS
ACT	PLU	28.22	13.97	1.37	273.20	24.85		40.92	22.52	
ACT	LI1	22.95	10.34	1.95	12.15	22.99		37.99	18.54	
ACT	LI2	33.50	7.65	2.50	23.53	19.42		44.86	12.60	TEST DATA
ACT	LI3	36.60	8.89	2.20	15.50	21.69		28.90	12.96	
ACT	LU1	26.93	8.51	2.12	6.37	25.13		46.75	28.84	
ACT	LU2	26.84	9.25	1.81	7.24	69.74	14.06	41.12	18.86	
ACT	LU3	24.74	9.70	2.09	7.59	32.75		43.83	28.74	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/15/76

SPECIES RHESUS/MONKEY COMPOUND 000138158

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	26.73	7.45	11.79	3.31	26.43	13.21	7.50	
ACT	A-C	25.84	9.64	7.98	6.21	27.12	16.19	7.53	NEGATIVE CONTROLS
ACT	ALI	30.06	6.89	18.45	10.77	56.55	15.71	7.72	
ACT	ALU	28.62	7.57	22.19	3.79	61.49	8.72	5.72	
ACT	PLI	60.22	58.15	3.07	580.84	88.51	67.41	24.55	POSITIVE CONTROLS
ACT	PLU	30.95	8.82	13.13	3.86	42.92	12.93	6.12	
ACT	LI1	29.48	8.94	43.37	10.94	37.97	10.97	4.08	
ACT	LI2	28.76	6.96	25.00	8.60	62.20	10.20	5.31	
ACT	LI3	33.81	6.59	22.84	13.51	45.01	13.45	4.99	TEST DATA
ACT	LU1	28.30	8.88	31.62	4.81	33.78	11.28	4.70	
ACT	LU2	29.47	7.14	20.25	5.97	29.98	10.45	6.61	
ACT	LU3	31.43	7.58	20.60	9.13	112.75	10.92	5.99	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI, ALU } or A+T = Activation: Homogenate Control (Liver, Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (*Macaca mulatta*)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, L-Glutamic Acid, HCL, FDA 75-59, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative. The LI1 dose with TA-1538 using mouse tissue, and the LU2 dose with TA-98 using rat tissue were repeated because of increased mutant frequencies. The repeat tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, L-Glutamic Acid, HCL, FDA 75-59, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

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Director
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Reviewed by:

Robert J. Weir, Ph.D. Date 3/31/77
Vice President

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his*$^-$ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

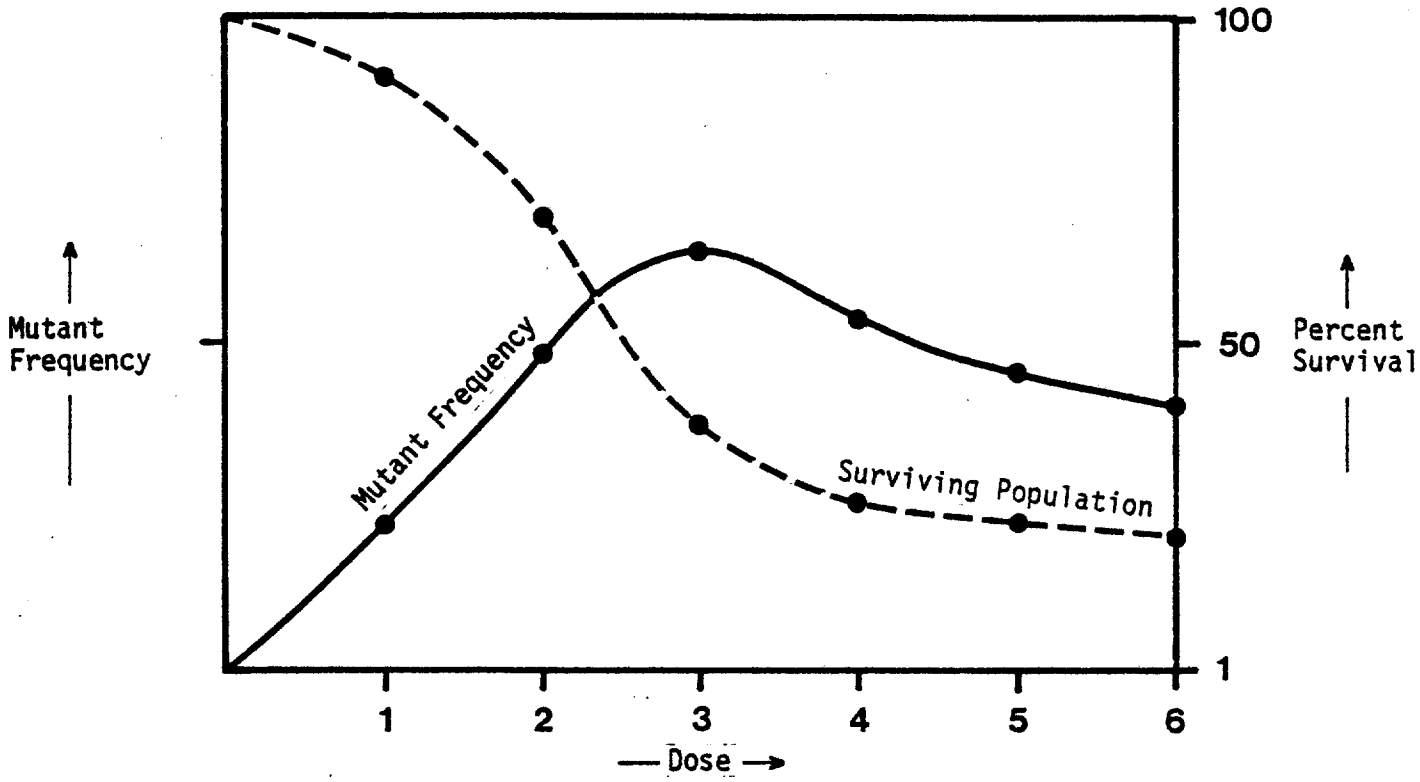
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627205 DETECTOR TA100 SPECIES / DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0803	0535	66.63	0
	NAP		EMS 0.066%	0548	4000	729.93	0
000138158	NA1		0025-3 PCT.	0907	0616	67.92	0
000138158	NA2		0125-4 PCT.	0835	0575	68.86	0
000138158	NA3		0625-5 PCT.	0880	0577	65.57	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 625801 DETECTOR TA1535 SPECIES / DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0257	0047	18.29	0
	NAP		EMS 0.2%	0081	4000	4938.27	0
000138158	NA1		0025-3 PCT.	0327	0026	7.95	0
000138158	NA2		0125-4 PCT.	0360	0036	10.00	0
000138158	NA3		0625-5 PCT.	0571	0064	11.21	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626501 DETECTOR TA1537 SPECIES / DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0221	0032	14.48	0
	NAP		QM 13 UG/ML	0251	0240	95.62	0
000138158	NA1		0025-3 PCT.	0719	0023	3.20	0
000138158	NA2		0125-4 PCT.	0342	0031	9.06	0
000138158	NA3		0625-5 PCT.	0350	0024	6.86	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626402 DETECTOR TA1538 SPECIES / DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0911	0011	1.21	0
	NAP		NF 667 UG/ML	0410	0590	143.90	0
000138158	NA1		0025-3 PCT.	0505	0009	1.78	0
000138158	NA2		0125-4 PCT.	0791	0010	1.26	0
000138158	NA3		0625-5 PCT.	0725	0012	1.66	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 625905 DETECTOR TA98 SPECIES / DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0202	0028	13.86	0
	NAP		NF 667 UG/ML	0049	0402	820.41	0
000138158	NA1		0025-3 PCT.	0756	0034	4.50	0
000138158	NA2		0125-4 PCT.	0420	0031	7.38	0
000138158	NA3		0625-5 PCT.	0411	0026	6.33	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 628801 DETECTOR 0000D4 SPECIES / DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	1118	0250	0109	22.36	9.75	0
	NAP		EMS 1.0 %	0294	0202	0112	68.71	38.10	0
000138158	NA1		0028-1 PCT.	1132	0203	0096	17.93	8.48	0
000138158	NA2		0014-1 PCT.	1218	0308	0124	25.29	10.18	0
000138158	NA3		0007-1 PCT.	1204	0120	0274	9.97	22.76	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627805 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	2342	0482	20.58	0
	A-C		SOLVENT	2274	0507	22.30	0
	ALI		TISSUE	2604	0636	24.42	0
	ALU		TISSUE	2104	0458	21.77	0
	ACP	LI	DMN 90 UM/ML	1400	0982	70.14	0
	ACP	LU	DMN 90 UM/ML	2506	0559	22.31	0
000138158	ACT	LI1	0025-3 PCT.	1970	0561	28.48	0
000138158	ACT	LI2	0125-4 PCT.	2522	0546	21.65	0
000138158	ACT	LI3	0625-5 PCT.	2294	0614	26.77	0
000138158	ACT	LU1	0025-3 PCT.	2362	0536	22.69	0
000138158	ACT	LU2	0125-4 PCT.	2182	0434	19.89	0
000138158	ACT	LU3	0625-5 PCT.	2408	0453	18.81	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 625904 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0488	0037	7.58	0
	A-C		SOLVENT	0539	0030	5.57	0
	ALI		TISSUE	0357	0023	6.44	0
	ALU		TISSUE	0383	0029	7.57	0
	ACP	LI	DMN 90 UM/ML	0353	0481	136.26	0
	ACP	LU	DMN 90 UM/ML	0337	0091	27.00	0
000138158	ACT	LI1	0025-3 PCT.	0217	0028	12.90	0
000138158	ACT	LI2	0125-4 PCT.	0405	0021	5.19	0
000138158	ACT	LI3	0625-5 PCT.	0299	0028	9.36	0
000138158	ACT	LU1	0025-3 PCT.	0210	0035	16.67	0
000138158	ACT	LU2	0125-4 PCT.	0458	0025	5.46	0
000138158	ACT	LU3	0625-5 PCT.	0471	0029	6.16	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629301 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0595	0016	2.69	0
	A-C		SOLVENT	0547	0032	5.85	0
	ALI		TISSUE	0518	0036	6.95	2
	ALU		TISSUE	0548	0020	3.65	0
	ACP	LI	AMQ 333 UG/ML	0311	0444	142.77	0
	ACP	LU	AMQ 333 UG/ML	0608	0013	2.14	0
000138158	ACT	LI1	0025-3 PCT.	0568	0039	6.87	0
000138158	ACT	LI2	0125-4 PCT.	0627	0035	5.58	0
000138158	ACT	LI3	0625-5 PCT.	0681	0019	2.79	0
000138158	ACT	LU1	0025-3 PCT.	0552	0034	6.16	0
000138158	ACT	LU2	0125-4 PCT.	0512	0039	7.62	0
000138158	ACT	LU3	0625-5 PCT.	0568	0040	7.04	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626001 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0954	0277	29.04	0
	A-C		SOLVENT	0862	0246	28.54	0
	ALI		TISSUE	0523	0260	49.71	0
	ALU		TISSUE	0924	0236	25.54	0
	ACP	LI	ANTH 67 UG/ML	0449	0910	202.67	0
	ACP	LU	ANTH 67 UG/ML	0842	0323	38.36	0
000138158	ACT	LI1	0025-3 PCT.	0577	0429	74.35	0
000138158	ACT	LI2	0125-4 PCT.	0602	0177	29.40	0
000138158	ACT	LI3	0625-5 PCT.	0609	0173	28.41	0
000138158	ACT	LU1	0025-3 PCT.	0565	0172	30.44	0
000138158	ACT	LU2	0125-4 PCT.	0630	0184	29.21	0
000138158	ACT	LU3	0625-5 PCT.	0658	0346	52.58	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626809 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	0441	0035	7.94	0
	ALU		TISSUE	0238	0029	12.18	0
000138158	ACT	LI1	0025-3 PCT.	0314	0035	11.15	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627206 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0657	0031	4.72	0
	A-C		SOLVENT	1003	0031	3.09	0
	ALI		TISSUE	0714	0071	9.94	0
	ALU		TISSUE	0702	0038	5.41	0
	ACP	LI	ANTH 67 UG/ML	0603	0641	106.30	0
	ACP	LU	ANTH 67 UG/ML	0770	0850	110.39	0
000138158	ACT	LI1	0025-3 PCT.	0469	0055	11.73	0
000138158	ACT	LI2	0125-4 PCT.	0544	0046	8.46	0
000138158	ACT	LI3	0625-5 PCT.	0418	0046	11.00	0
000138158	ACT	LU1	0025-3 PCT.	0495	0048	9.70	0
000138158	ACT	LU2	0125-4 PCT.	0455	0042	9.23	0
000138158	ACT	LU3	0625-5 PCT.	0607	0047	7.74	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629202 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1474	0343	0120	23.27	8.14	0
	A-C		SOLVENT	1304	0341	0104	26.15	7.98	0
	ALI		TISSUE	1244	0330	0108	26.53	8.68	0
	ALU		TISSUE	1194	0345	0103	28.89	8.63	0
	ACP	LI	DMN 90 UM/ML	0848	0574	0232	67.69	27.36	0
	ACP	LU	DMN 90 UM/ML	1111	0360	0127	32.40	11.43	0
000138158	ACT	LI1	0028-1 PCT.	1224	0328	0147	26.80	12.01	0
000138158	ACT	LI2	0014-1 PCT.	1220	0327	0142	26.80	11.64	0
000138158	ACT	LI3	0007-1 PCT.	0952	0235	0105	24.68	11.03	0
000138158	ACT	LU1	0028-1 PCT.	1240	0358	0131	28.87	10.56	0
000138158	ACT	LU2	0014-1 PCT.	1234	0302	0116	24.47	9.40	0
000138158	ACT	LU3	0007-1 PCT.	0970	0286	0108	29.48	11.13	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627801 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	2500	0518	20.72	0
	A-C		SOLVENT	2288	0591	25.83	0
	ALI		TISSUE	2172	0683	31.45	0
	ALU		TISSUE	2008	0543	27.04	0
	ACP	LI	DMN 90 UM/ML	1419	0876	61.73	0
	ACP	LU	DMN 90 UM/ML	2254	0636	28.22	0
000138158	ACT	LI1	0025-3 PCT.	3334	0765	22.95	0
000138158	ACT	LI2	0125-4 PCT.	2364	0792	33.50	0
000138158	ACT	LI3	0625-5 PCT.	2150	0787	36.60	0
000138158	ACT	LU1	0025-3 PCT.	2280	0614	26.93	2
000138158	ACT	LU2	0125-4 PCT.	2016	0541	26.84	0
000138158	ACT	LU3	0625-5 PCT.	1940	0480	24.74	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626701 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0909	0098	10.78	0
	A-C		SOLVENT	1063	0108	10.16	0
	ALI		TISSUE	0672	0091	13.54	0
	ALU		TISSUE	0823	0104	12.64	2
	ACP	LI	DMN 90 UM/ML	1272	3140	246.86	0
	ACP	LU	DMN 90 UM/ML	0687	0096	13.97	2
000138158	ACT	LI1	0025-3 PCT.	0861	0089	10.34	0
000138158	ACT	LI2	0125-4 PCT.	0758	0058	7.65	0
000138158	ACT	LI3	0625-5 PCT.	0675	0060	8.89	0
000138158	ACT	LU1	0025-3 PCT.	0646	0055	8.51	2
000138158	ACT	LU2	0125-4 PCT.	0638	0059	9.25	2
000138158	ACT	LU3	0625-5 PCT.	0670	0065	9.70	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629501 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0600	0025	4.17	0
	A-C		SOLVENT	0641	0017	2.65	0
	ALI		TISSUE	0669	0025	3.74	1
	ALU		TISSUE	0568	0012	2.11	0
	ACP	LI	AMQ 333 UG/ML	0200	0245	122.50	1
	ACP	LU	AMQ 333 UG/ML	0582	0008	1.37	2
000138158	ACT	LI1	0025-3 PCT.	0512	0010	1.95	1
000138158	ACT	LI2	0125-4 PCT.	0519	0013	2.50	0
000138158	ACT	LI3	0625-5 PCT.	0546	0012	2.20	0
000138158	ACT	LU1	0025-3 PCT.	0565	0012	2.12	0
000138158	ACT	LU2	0125-4 PCT.	0551	0010	1.81	0
000138158	ACT	LU3	0625-5 PCT.	0573	0012	2.09	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626505 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0427	0021	4.92	0
	A-C		SOLVENT	0486	0020	4.12	0
	ALI		TISSUE	0271	0050	18.45	0
	ALU		TISSUE	0374	0028	7.49	0
	ACP	LI	ANTH 67 UG/ML	0269	0588	218.59	0
	ACP	LU	ANTH 67 UG/ML	0250	0683	273.20	0
000138158	ACT	LI1	0025-3 PCT.	0247	0030	12.15	2
000138158	ACT	LI2	0125-4 PCT.	0102	0024	23.53	0
000138158	ACT	LI3	0625-5 PCT.	0129	0020	15.50	0
000138158	ACT	LU1	0025-3 PCT.	0377	0024	6.37	0
000138158	ACT	LU2	0125-4 PCT.	0290	0021	7.24	0
000138158	ACT	LU3	0625-5 PCT.	0369	0028	7.59	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626502 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1712	0272	15.89	0
	A-C		SOLVENT	2147	0267	12.44	0
	ALI		TISSUE	2557	0297	11.62	0
	ALU		TISSUE	1650	0331	20.06	0
	ACP	LI	ANTH 67 UG/ML	1116	0938	84.05	0
	ACP	LU	ANTH 67 UG/ML	1304	0324	24.85	0
000138158	ACT	LI1	0025-3 PCT.	0970	0223	22.99	0
000138158	ACT	LI2	0125-4 PCT.	1442	0280	19.42	0
000138158	ACT	LI3	0625-5 PCT.	0982	0213	21.69	0
000138158	ACT	LU1	0025-3 PCT.	1138	0286	25.13	0
000138158	ACT	LU2	0125-4 PCT.	1117	0779	69.74	0
000138158	ACT	LU3	0625-5 PCT.	0913	0299	32.75	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629303 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	ALI		TISSUE	0920	0148	16.09	0
	ALU		TISSUE	0922	0140	15.18	0
000138158	ACT	LU2	0125-4 PCT.	0953	0134	14.06	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629502 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0886	0381	0191	43.00	21.56	0
	A-C		SOLVENT	0761	0390	0155	51.25	20.37	0
	ALI		TISSUE	0708	0296	0187	41.81	26.41	0
	ALU		TISSUE	0726	0284	0153	39.12	21.07	0
	ACP	LI	DMN 90 UM/ML	0567	0453	0343	79.89	60.49	0
	ACP	LU	DMN 90 UM/ML	0826	0338	0186	40.92	22.52	0
000138158	ACT	LI1	0028-1 PCT.	0874	0332	0162	37.99	18.54	0
000138158	ACT	LI2	0014-1 PCT.	0778	0349	0098	44.86	12.60	0
000138158	ACT	LI3	0007-1 PCT.	0872	0252	0113	28.90	12.96	0
000138158	ACT	LU1	0028-1 PCT.	0631	0295	0182	46.75	28.84	0
000138158	ACT	LU2	0014-1 PCT.	0822	0338	0155	41.12	18.86	0
000138158	ACT	LU3	0007-1 PCT.	0689	0302	0198	43.83	28.74	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627901 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	1736	0464	26.73	0
	A-C		SOLVENT	1842	0476	25.84	0
	ALI		TISSUE	2708	0814	30.06	0
	ALU		TISSUE	2442	0699	28.62	0
	ACP	LI	DMN 90 UM/ML	1184	0713	60.22	0
	ACP	LU	DMN 90 UM/ML	2430	0752	30.95	0
000138158	ACT	LI1	0025-3 PCT.	2422	0714	29.48	0
000138158	ACT	LI2	0125-4 PCT.	2510	0722	28.76	0
000138158	ACT	LI3	0625-5 PCT.	2156	0729	33.81	0
000138158	ACT	LU1	0025-3 PCT.	2336	0661	28.30	0
000138158	ACT	LU2	0125-4 PCT.	2470	0728	29.47	0
000138158	ACT	LU3	0625-5 PCT.	2466	0775	31.43	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 628001 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	2201	0164	7.45	0
	A-C		SOLVENT	2313	0223	9.64	0
	ALI		TISSUE	2757	0190	6.89	0
	ALU		TISSUE	2683	0203	7.57	0
	ACP	LI	DMN 90 UM/ML	1754	1020	58.15	0
	ACP	LU	DMN 90 UM/ML	2777	0245	8.82	0
000138158	ACT	LI1	0025-3 PCT.	2518	0225	8.94	0
000138158	ACT	LI2	0125-4 PCT.	2642	0184	6.96	0
000138158	ACT	LI3	0625-5 PCT.	2578	0170	6.59	0
000138158	ACT	LU1	0025-3 PCT.	2500	0222	8.88	0
000138158	ACT	LU2	0125-4 PCT.	2507	0179	7.14	0
000138158	ACT	LU3	0625-5 PCT.	2558	0194	7.58	3

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629701 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	2774	0327	11.79	2
	A-C		SOLVENT	1818	0145	7.98	2
	ALI		TISSUE	0981	0181	18.45	2
	ALU		TISSUE	0996	0221	22.19	2
	ACP	LI	AMQ 333 UG/ML	2119	0065	3.07	2
	ACP	LU	AMQ 333 UG/ML	2400	0315	13.13	2
000138158	ACT	LI1	0025-3 PCT.	0498	0216	43.37	0
000138158	ACT	LI2	0125-4 PCT.	0828	0207	25.00	0
000138158	ACT	LI3	0625-5 PCT.	0893	0204	22.84	0
000138158	ACT	LU1	0025-3 PCT.	0816	0258	31.62	0
000138158	ACT	LU2	0125-4 PCT.	1210	0245	20.25	0
000138158	ACT	LU3	0625-5 PCT.	1330	0274	20.60	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 627104 CONTRACT 22374-2104 DETECTOR TA1538 PROJECT 02468 SPECIES RHESUS/MONKEY DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0544	0018	3.31	0
	A-C		SOLVENT	0306	0019	6.21	0
	ALI		TISSUE	0418	0045	10.77	0
	ALU		TISSUE	0554	0021	3.79	0
	ACP	LI	ANTH 67 UG/ML	0334	1940	580.84	0
	ACP	LU	ANTH 67 UG/ML	0674	0026	3.86	0
000138158	ACT	LI1	0025-3 PCT.	0192	0021	10.94	0
000138158	ACT	LI2	0125-4 PCT.	0186	0016	8.60	0
000138158	ACT	LI3	0625-5 PCT.	0185	0025	13.51	0
000138158	ACT	LU1	0025-3 PCT.	0291	0014	4.81	0
000138158	ACT	LU2	0125-4 PCT.	0201	0012	5.97	0
000138158	ACT	LU3	0625-5 PCT.	0219	0020	9.13	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627401 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1559	0412	26.43	1
	A-C		SOLVENT	1803	0489	27.12	0
	ALI		TISSUE	0916	0518	56.55	0
	ALU		TISSUE	0753	0463	61.49	1
	ACP	LI	ANTH 67 UG/ML	1088	0963	88.51	1
	ACP	LU	ANTH 67 UG/ML	0953	0409	42.92	0
000138158	ACT	LI1	0025-3 PCT.	1114	0423	37.97	0
000138158	ACT	LI2	0125-4 PCT.	0709	0441	62.20	0
000138158	ACT	LI3	0625-5 PCT.	0871	0392	45.01	0
000138158	ACT	LU1	0025-3 PCT.	1181	0399	33.78	0
000138158	ACT	LU2	0125-4 PCT.	1304	0391	29.98	1
000138158	ACT	LU3	0625-5 PCT.	0353	0398	112.75	1

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629503 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 11/15/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0787	0104	0059	13.21	7.50	0
	A-C		SOLVENT	0624	0101	0047	16.19	7.53	0
	ALI		TISSUE	0751	0118	0058	15.71	7.72	0
	ALU		TISSUE	0734	0064	0042	8.72	5.72	0
	ACP	LI	DMN 90 UM/ML	0721	0486	0177	67.41	24.55	0
	ACP	LU	DMN 90 UM/ML	0735	0095	0045	12.93	6.12	0
000138158	ACT	LI1	0028-1 PCT.	0711	0078	0029	10.97	4.08	0
000138158	ACT	LI2	0014-1 PCT.	0716	0073	0038	10.20	5.31	0
000138158	ACT	LI3	0007-1 PCT.	0721	0097	0036	13.45	4.99	0
000138158	ACT	LU1	0028-1 PCT.	0745	0084	0035	11.28	4.70	0
000138158	ACT	LU2	0014-1 PCT.	0756	0079	0050	10.45	6.61	0
000138158	ACT	LU3	0007-1 PCT.	0751	0082	0045	10.92	5.99	0

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.